Notes on ....

Podocyte complexity and terrestriality in frogs

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Abstract. We extend Richter & Splechtna's (1996) study of the morphology of amphibian podocytes with respect to habitat aridity and water regulation, and describe the method for calculating complexity index of podocyte pedicels. Our results show that *Notaden nichollsi*, the most terrestrial species examined so far, has the highest podocyte complexity. The relationship between podocyte complexity and habitat suggests that glomerular filtration rate is an important parameter related to habitat aridity and water regulation in frogs.

Introduction

Amphibians are found in habitats ranging from fully aquatic to fully terrestrial and sometimes extremely arid. The permeable skin of most amphibians allows substantial trans-cutaneous water and solute flux, and frogs have an effective water regulation system comprising a very distinct lymphatic system, kidneys and bladder (Steen 1929; Ewer 1952; Bentley *et al.* 1958; Middler *et al.* 1968; Bentley 1969; Shoemaker & Nagy 1977; Shoemaker *et al.* 1992).

Water regulation by frogs is determined in part by glomerular filtration rate (GFR), the rate of water entry into the lumen of the renal tubules (Heller 1950). GFR is determined morphologically by the microstructure of podocyte cells that cover glomerular capillaries, as well as physiological factors such as plasma hydrostatic and colloid osmotic pressure. The pedicels (foot-like projections) of podocytes form filtration slits, the number and size of which influences GFR.

Richter & Splechtna (1996) used scanning electron microscopy to examine the complexity of podocytes for a variety of frogs differing in terrestriality. They distinguished five types of podocyte process systems, and suggested that podocyte pedicel microstructure was correlated with habitat *i.e.* podocyte complexity increases with terrestriality. Aquatic frogs have fewer high-branching levels than terrestrial species. We examine podocyte complexity for the Australian frog *Notaden nichollsi*, and *Xenopus laevis* and *Bufo marinus*, using a similar protocol to Richter & Splechtna (1996).

Materials and Methods

A desert spadefoot toad (*Notaden nichollsi*) and a cane toad (*Bufo marinus*) were killed by double pithing, and a perfused and fixed South African clawed toad (*Xenopus laevis*) was obtained from other experiments. Frog kidneys were perfusion-fixed with 4% paraformaldehyde in cacodylate buffer and post-fixed with osmium-thiocarbohydrazide (Richter & Splechtna 1996). They were then mounted on SEM stubs and critical point dried. A Philips 505 scanning electron microscope was used to locate glomeruli on the kidney surface, then a Field Emission Scanning Electron Microscope JSM 6300F was used for higher resolution imaging.

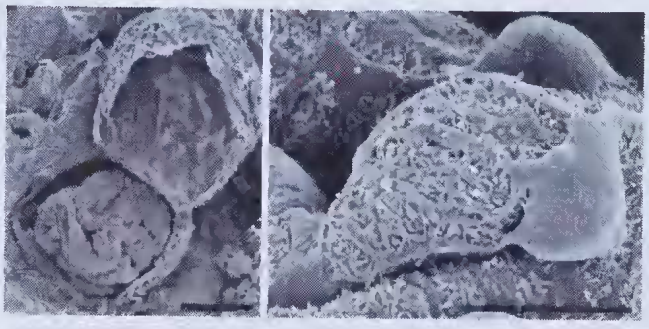


Figure 1. High resolution photomicrographs of *Bufo marinus* podocytes showing glomerulus within a Bowman's capsule (left; scale bar 50 µm) and podocytes on the glomerular capillaries (right; scale bar 10 µm).

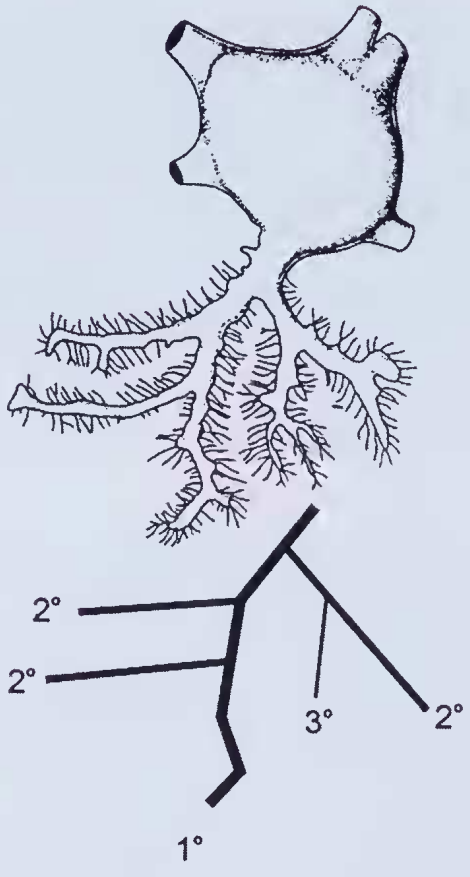
Podocytes on the surface of glomeruli in the kidney of frogs were difficult to locate, and it was also difficult to obtain photographs of the podocytes with sufficient resolution for clear definition of the branching patterns. Nevertheless, we obtained photomicrographs (*e.g.* Fig 1) for the calculation of podocyte complexity for three species, the aquatic *Xenopus laevis* (n =1 individual, 3 glomeruli, 5 podocytes), the mesic terrestrial *Bufo marinus* (n = 1; 1 glomerulus, 3 podocytes) and the arid terrestrial *Notaden nichollsi* (n = 1; 1 glomerulus, 2 podocytes).

Podocyte complexity index

Richter & Splechtna (1996) did not provide any detail of the methodology for calculating the index for podocyte complexity, but merely cited Laskowski & Pohlit (1974), a biophysical book published only in German. So, we describe here our interpretation of the methodology required to replicate Richter & Splechtna's (1996) calculations.

Firstly, the number of branches in each branching level (from 1 to 4) of a podocyte cell body is determined. Primary level podocyte processes branch out to secondary level processes, and then to tertiary and quaternary levels. In determining branch levels in each podocyte group, the main branch always retains the status of the previous branch level. The main branch is defined as the initial branch extending from the cell body, and with the continuation that provides the maximum branching complexity.

After the branching levels are determined, the total number of branch endings is counted, and the fraction (P_i) of branch endings belonging to each branch level is calculated. For each group, P_i is multiplied by the branch level (Z_i), and the complexity index (bit value) for each podocyte cell body is calculated by summing the P_iZ_i values. The complexity index is defined such that the higher the bit value, the more complex the structure is. An example of how to determine the branching levels of



Total number of branch endings = 5

	P_i	Z_i	P_iZ_i
1°	1/5	1	1/5
2°	3/5	2	6/5
3°	1/5	3	3/5
			10/5 = 2.0 bit

Figure 2. Example of calculation of podocyte complexity index for branching level for podocyte pedicels (type III, from Richter & Splechtna 1996) using schematic of primary pedicel branch (1°), secondary branches (2°) and tertiary branch (3°).

the processes of podocytes is shown in Fig 2. The podocyte complexity index for an individual podocyte is determined by taking the average SP_iZ_i value for several randomly selected podocyte cell bodies.

Results and Discussion

The average complexity index obtained for pedicels of podocytes from *X. laevis* was low, at 1.27 bits; it was 2.32 bits for *B. marinus;* and was highest (2.46 bits) for *N. nichollsi*. The bit value obtained for *X. laevis* was very close to that of Richter & Splechtna (1996). Direct comparison between the complexity indices obtained in this study with those of Richter & Splechtna (1996) shows a strong relationship between podocyte pedicel complexity and terrestriality (Fig 3), presumably mediated by variation in glomerular filtration rate.

The podocyte pedicel index for *N. nichollsi*, the most arid terrestrial amphibian examined so far, is also the highest value measured, conforming well with the pattern established by Richter & Splechtna (1996). Further studies of additional species will clearly be productive in more fully documenting the relationship between podocyte microstructure, kidney function and water balance for the many and diverse species of anuran amphibians.

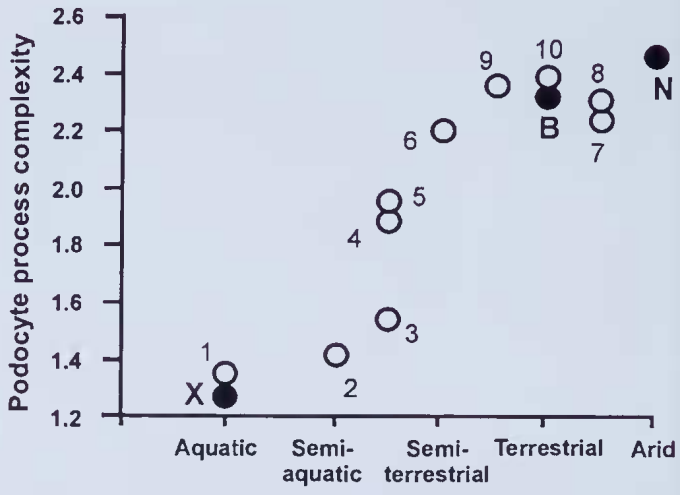


Figure 3. Relationship between habitat and podocyte complexity (adapted from Richter & Splechtna 1996). Values from the present study are *Xenopus laevis* (X; 1.27 bits), *Bufo marinus* (B; 2.32 bits) and *Notaden nichollsi* (N; 2.46 bits). Species from Richter & Splechtna (1996) are; 1, *Xenopus laevis*; 2, *Rana ridibunda*; 3, *Rana esculenta*; 4, *Bombina variegata scabra*; 5, *Discoglossus pictus*; 6, *Rana lessonae*; 7, *Alytes cysternasii*; 8, *Bufo regularis*; 9, *Rana temporaria*; 10, *Rana dalmatia*.

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References

Bentley PJ, Lee AK & Main AR 1958 Comparison of dehydration and hydration of two genera of frogs (*Heleioporus* and *Neobatrachus*) that live in areas of varying aridity. Journal of Experimental Biology 35: 677-684.

Bentley PJ 1969 Neurohypophysial function in Amphibia: hormone activity in the plasma. Journal of Endocrinology 43: 359-369.

Ewer RF 1952 The effects of posterior pituitary extracts on water balance in *Bufo carens* and *Xenopus laevis*, together with some general considerations of anuran water economy. The Journal of Experimental Biology 29:429-439.

Heller H 1950 The comparative physiology of the neurohypophysis. Experientia 6:368-376.

Laskowski W & Pohlit W 1974 Biophysik. Bd 1: Struktur, Energie, Information und Bausteine belebter Systeme. George Thieme Verlag, Stuttgart.

Middler SA, Kleeman C R & Edwards E 1968 Lymph mobilization following acute blood loss in the toad, *Bufo marinus*. Comparative Biochemistry and Physiology 24:343-353.

Richter S & Splechtna H 1996 The structure of anuran podocyte — determined by ecology? Acta Zoologica 77:335-348.

Shoemaker VH & Nagy KA 1977 Osmoregulation in amphibians and reptiles. Annual Review of Physiology 39:449-571.

Shoemaker VH, Hillman SS, Hillyard SD, Jackson DC, McClanahan LL, Withers PC & Wygoda ML 1992 Exchange of water, ions, and respiratory gases in terrestrial amphibians. In: Environmental Physiology of the Amphibians (eds M E Feder & W W Burggren). University of Chicago Press, Chicago, 125-150.

Steen WB 1929 On the permeability of the frogs' bladder to water. Anatomical Record 43:215-220.